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Histoplasmosis, (U)

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HISTOPLASMOSIS

Mycotic diseases, and especially organic mycoses, are beginning to
attract, more frequently, the attention of the medical world. There are at
least three important reasons which explain this fact. First, the classical
infections caused by bacteria, spirilla, or Rickettsia, have been already
sufficiently identified as to epidemiology as well as therapy. Secondly,
in the era of common application of chemo-therapeutics and antibiotics,
arises the question of frequent complications of diseases which have bacterial
etiology, as well as other diseases, through pathogenetic fungi. Eventually
it became known that the role of fungi - of potential pathogenetic factors
commonly found in nature - has been, until now, underestimated. A good
example illustrating the latter is histoplasmosis. At first it was considered
to be a rare disease. Today, it proves to be an epidemiological disease,
affecting millions of people.

Histoplasma Capsulatum was discovered by Darling (20) in Panama, in
1906, in the course of a study of leishmaniosis. Darling first included
Histoplasma Capsulatum into protozoa, then he began to agree with the opinion
of Da Rocha Lima (21) who, comparing Darling's cases with the kala-azar
disease and the inflammation of lymphatic vessels in soliped animals,
ascertained, that Histoplasma Capsulatum is similar to Cryptococcus
Farciminosis, i.e. to the etiological factor, the above mentioned disease of
solipeds, and belongs to fungi. This was finally explained by examinations of
De Monbreun (23), as well as Hansman and Schenken (43) who, in the case of
human histoplasmosis, have isolated Histoplasma Capsulatum on an artificial
medium and confirmed its belonging to imperfect fungi. From that moment
cases of histoplasmosis were more frequently observed, thus confirming the
identification through isolation of micro-organisms. Nevertheless, it was

presumed, that the infections, caused by Histoplasma Capsulatum, are un-
usually rare and do not have significant epidemiological meaning. A break-
through came with the discoveries made by American scientists (6, 12, 35,
67, 73) and others, who found out that Histoplasma Capsulatum is responsible
for many diseases of, until then, unknown etiology.(36,52).

Within the last decade the wide-spread presence of Histoplasma
Capsulatum has been finally confirmed as well as the immensely frequent
infection caused by this micro-organism embracing, for example, in some
states of the U.S.A. about 80% of the population (27, 37, 41, 57). It has
been confirmed, in the majority of cases, that the infections do not show
any symptoms in their course, or they are in sub-clinical form frequently
leaving characteristic calcification within the lungs or in other internal
organs which is similar to tuberculous calcifications.

Epidemiology and the Spread of Histoplasmosis

For a long time the appearance of fungus in nature was unknown.
Only in 1949 Emmons (30) accomplished the isolation of Histoplasma
Capsulatum from the soil in an endemic area. This discovery, which was
later repeated many times by other scientists (2, 51, 56), determined,
finally, the role of the soil as a natural environment for Histoplasma
Capsulatum. Subsequently, many positive cultures of fungi were developed
from the air (49), water (39), dust, chicken lodgings, rotten wood (44),
old silos (40), from the areas of old, abandoned houses occupied by pi-
geons and bats (32,33); as well as from many domestic and wild animals.
(1,3,13-15,24,31).

Histoplasmosis has been discribed in many animal species, yet the
immediate spread of infection from a diseased animal to a healthy one, or
or from an animal to a human being, has not been confirmed. It follows that
such transmissions do not take place at all or they occur unusually seldom,
and they don't play a substantive role in epidemiology.

The infection develops as result of the fungus invasion into the
organism through respiratory or alimentary organs. (57). Histoplasmosis
has been also observed in humans; it resulted from penetration of the
parasite through damaged skin or through mucous membranes. There is a
great probability that the transmission of infection from an infected
organism to a healthy one can occur through the medium of ticks (65).

The infections caused by Histoplasma Capsulatum materialize in
humans regardless of race, age or sex; however, they develop more fre-
quently in children than in adults. The development of histoplasmosis in
childhood has been observed, equally often, in both sexes; from 40 to 60
years of age mycosis appears more frequently in men than in women. It

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has been also confirmed that the cases of histoplasmosis are slightly more numerous among the agricultural population than among the city dwellers.

Histoplasmosis has been observed in all continents. Quite frequently however, this disease is found in North and South America, especially in North America. Into endemic areas are included mid-west and north-eastern parts of U.S.A. The cases of histoplasmosis have been also observed in in South and West Africa, Sudan, south-eastern Asia, in Malaya Archipelago, Philippines, Australia, and in the following European countries: England, Austria, Bulgaria, France, Spain and Portugal.

As an indicator of the frequency of infections caused by Histoplasma Capsulatum serves the allergical skin test performed with histoplasmin. This reaction, which was for the first time applied by Van Pernis and collaborators (84), made possible the examination of large groups of people. It demonstrated, in some countries, for example U.S.A., areas of endemic histoplasmosis. The tests with histoplasmin, carried out in other countries, were of a much smaller scope and, therefore, can not truly reflect the state of infection through Histoplasma Capsulatum.

In Poland the skin tests with histoplasmin were conducted in 1952 by a team from WHO Tuberculosis Research Office. Two groups of school children were examined. In the city of Krakow only one positive result was obtained out of 596 examined children. The infected child was a seven years old boy. In Siedlice all the tests performed on 396 children showed negative results. (27, 60).

In conducting tests with histoplasmin among large groups of people it was possible, beyond any doubt, to discover a connection between histo- plasmin positiviness and the presence of calcification in the lungs of individuals showing a negative tuberculine reaction. Thanks to numerous studies (12, 64, 66, 72, 73, 82, 86, 88, 89), in some states of U.S.A., for example, frequent cases of calcification centers in lungs have been established, formed because of the histoplasmic infection and not because of tuberculosis (26, 28). Thus, it became possible to solve the intriguing problem of the etiology of changes (lesions) in lungs, similar to tuber- culosis, with negative inoculation of tuberculous bacilli and the lack of tuberculine allergy.

It is appropriate here to emphasize the great significance which the discoveries in the field of other organic mycosis – coccidioidomycosis.– had for the study of histoplasmosis. Due to the fact that coccidioidomycosis is, in its many attributes, similar to histoplasmosis and that the studies of coccidioidomycosis have been made somewhat earlier, it was possible to avoid errors and unnecessary research in explaining the problems of histo- plasmosis.

Pathogenetic Factor

Histoplasma Capsulatum belongs to imperfect fungi, which appear in two phases: 1) the yeasty, tissue phase, called by American authors YP (Yeast Phase), and 2) the mycotic phase, also called MP (Mycelial phase). In an infected organism, micro-organism appears exclusively in the yeasty phase, although cases have been described in which the presence of mycotic phase in infected tissues was confirmed.(42).

The yeasty phase of Histoplasma Capsulatum appears, under microscope, in the form of oval cells in diameter of 1 to 4 u, which are similar to yeast cells. It is possible to observe in the infected tissue, around the cells of Histoplasma Capsulatum, translucent zones, sharply refracting light, which made it possible for Darling (20) to confirm the presence of a capsule and thus give the name of "capsulatum". Later examinations, using an electronic microscope, did not confirm existence of a capsule. (74).

Histoplasma Capsulatum, in the mycotic phase, has the form of mycelium. It is formed from shreds in diameter of 2,5 u, possessing ramifications and forming on extremities or on the course of threads, so-called chlamydospores. A characteristic feature of the mycotic phase are so-called "tuberculous chlamydospores" (tuberculate chlamydospores). They are round, large, or oval spores, in diameter of 7-8 u, possessing on the swollen membrane numerous tubercular stripes.

On artificial media both phases show a different type of growth. The yeasty phase grows in constant media in the form of round, smooth, glossy white colonies of medium size; the mycotic phase has the appearance of dry, flat, irregular, sprinkled colonies, which are white at the beginning and after a dozen or so days they turn yellow-brown.

The cultures of Histoplasma Capsulatum are developed in quite a great range of temperatures: 20° to 37°. To procure the growth of the yeasty phase the 37° temperature is indispensable. The reaction of the medium is somewhat acid, fluctuating from pH6 to pH7. The growth takes place in oxygen conditions, though it is helpful to increase the access of CO_2 (15-20% CO_2). To the most frequently used constant media belong Sauranda media containing 2-4% of glucose, and media containing grain starch. The latter serve in establishing the presence of tuberculous chlamydospores.

It can be seen from the studies of Pinea (70) that media containing agar do not always constitute a suitable substratum for growth. Pinea found out that fatty acids, which are in agar, are inhibitors of Histoplasma Capsulatum. The activity of these acids can be abolished with the help of compounds containing SH groups in the reduced form (ex.: cysteine, gluthathione). Certain blood, and especially red blood corpuscles, show a clear influence by stimulating the growth of Histoplasma Capsulatum.

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Histoplasma Capsulatum multiplies through transversal partition or through budding, creating blastospores or chlamydospores. There are tuberculous chlamydospores as well as smaller ones, in diameter 2,5 to 4 u, having thin and long walls. Both kinds of chlamydospores can develop directly from shreds or they come into being on conidiophores.

Passing from the mycotic phase into the yeasty phase, and vice versa, depends on the conditions of environment. The reversion into mycotic phase occurs in natural conditions. To preserve the micro-organism in the yeasty phase or to develop it from the mycotic into yeasty phase, is a difficult task requiring complicated media and, sometimes, the only effective mean is the passage through a living organism. A good medium for passing from the mycotic phase into the yeasty phase is the medium of composition given by Littman (55). It contains liver and spleen extracts, human blood, glucose, agar, and antibiotics.

The antigenic structure of Histoplasma Capsulatum is relatively little known. The majority of studies were concentrated on the problem of antigenic affinity with other pathogenic, diphase fungi like: coccidioides immitis, Blastomyces dermatitidis, Candida albicans, etc. In making examinations, the fixation reaction, precipitation, passive hemagglutination and allergic reaction, were used. Agglutinative reaction can not be applied on account of spontaneous agglutination of Histoplasma Capsulatum cells.

Histoplasma Capsulatum has, in contrast to the majority of pathogenetic fungi, relatively strong antigenic characteristics. The method of obtaining immuno sera was gradually improving. At first, experimental animals were immunised during a period lasting several months; now, good sera can be obtained by applying large doses of antigen in short intervals of time, i.e. 3-5 weeks (76).

The studies of antigenic affinity of deep mycoses etiological factors showed that Histoplasma Capsulatum contains some antigenic fractions in common with Coccidioides immitis, Blastomyces dermatitidis and Candida albicans. The description of mutual antigenic affinity between above mentioned micro-organisms explained many questions connected with diagnostics of pathogenetic, diphase fungi.

Histoplasmosis in Humans

The course of histoplasmic infection in man shows a great variety. It depends on many factors such as predisposition, age, the state of natural immunity, the intensity of infection, etc. As result of the infection developing most frequently per os; and only sporadically through damaged skin, a primary complex is formed future fate of which can be various. In the majority of cases the infection, subjectively and objectively, is asymptomatic. Only in a certain goup of people suffering from such form of the disease, the changes remain in the form of a single one or several centers of

calcification within the area of lungs or spleen. The only certain gauge of
infection that passed in mild, asymptomatic forms, is the state of produced
allergy to histoplasmin.

In symptomatic forms the changes and symptoms of the disease are of
unusually great variety and, therefore, create much difficulty in the
formation of individual clinical complexes. On the basis of Wilson's study,
(85), who attempted to establish the clinical forms on the bases founded
in other mycosis - coccidioidomycosis - following complexes can be singled
out:
    1. primary histoplasmosis of the skin
    2. primary histoplasmosis of the lungs
    3. disseminated histoplasmosis.
The first form, the primary histoplasmosis of the skin, appears sporadically
and is characterized by forming of ulceration on the spot of infection as well
as by the inflammation of surrounding vessels and lymphatic nodes. A
similar case, described by Curtis and Cawley (19), ended favorably; in the
body of the diseased no other histoplasmic changes were noted. The
individual, in the course of further observation, did not show recurrence
of the disease.

The primary histoplasmosis of the lungs can develop in bronchial
or blood vessel tracts. On the spot where the germ settles a reaction is
forming which resembles the picture of tuberculous deposit incrustation.
The inflammatory process, produced in pulmonic membrane, can have various
exits. It can lead to complete resorption, incrustation, disintegration
with the creation of a cavity, fibrosis, calcification, or formation of
histoplasmin. The inflammatory changes can be numerous, and then, in
unfavorable cases, it leads to fibrosis of the entire segments of lungs,
emphysema and bronchial ectasis. Histoplasmic process attacks, sometimes,
pleura. Then comes to inflammation of pleura with formation of exudate
(4). The mucios membranes of upper respiratory channels also succumb,
quite frequently, to pathologic process, whether as result of primary or
secondary nestling of the bacillus. The cases of histoplasmosis of the
mouth cavity, throat, and larynx have been described; the changes in
these cases had the ulcerative character, sometimes imitating neoplastic
tissue (5, 18, 62). To subjective symptoms, which appear in this
complex, belong typical complaints about diseases of respiratory organs.
The primary histoplasmosis of the lungs encompasses not only symptomatic
forms, but also frequent asymptomatic cases.

The disseminated histoplasmosis can occur, like the preceding
complex, in symptomatic or asymptomatic forms, though asymptomatic
course is rather infrequent. As result of the generalization of in-
fection a characteristic picture is being formed: the reticular- endo-
thelious system is occupied which is manifested by hyperplasia of the
spleen, liver or lymphatic glands. This form is accompied by symptoms of

...emia, changes in peripheral blood, anemia, leukopenia, fever, disturbances in alimentary canal in the form of vomiting, diarrhea, bleeding in stomach and intestines, formed because of ulceration and perforation. As result of dissemination of infection many internal organs are impaired. Frequent are changes within the liver and in suprarenal body, causing their insufficiency.

The course of the disseminated histoplasmosis is usually fatal. Acute cases end in death within few weeks, lingering cases can last even 18 to 20 years. The generalized form materializes in individuals demonstrating certain immunological defects in regard to Histoplasma Capsulatum infections, which does not let them to develop normal defensive mechanisms.

Interesting is frequent coexistance or joining of histoplasmosis with diseases in which the disturbances in reticular endothelious system are confirmed; for example, in various forms of leukemia, in Hodgkins disease, or in lymphosarcoma. This phenomenon can be explained doubly: it is possible, that as the result of primary damage to hematopoietic system and RES the resistance powers of the organism are insufficient to liquidate histoplasmic infection or, conversely, as result of the infection develops a reaction of these tissues which reminds of lymphoblastomatic states. (85).

The Histopathological Picture of Histoplasmosis

The majority of scientists agree that the histopathological picture of histoplasmosis is similar to tuberculosis. In the form of disseminated histoplasmosis, or pulmonary histoplasmosis there appear, within lymphatic glands, producing changes showing tendency for disintegration. In the lungs, which are almost always attacked, miliary tubercles are formed and the lymphatic glands are swelling. Within the liver and spleen, which are enlarged, hyperemic, appear tubercles and necrotic area. Bone-marrow and suprarenal glands are also frequently involved in the disease process.

According to Wilson (85), one can observe in the changed tissues the appearance of Histoplasma Capsulatum within the cells of the RES system, in the form of round or oval formations, surrounded by translucent zones imitating an areola. Here and there are visible germating cells. The concentrations of parasites within tubercles are surrounded by pseudo-tuberculous tissue, composed of lymphocytes, plasmatic cells, fibroblasts, macrophags, epithelioid cells, and macro-cells. The combining of such centers can cause the formation of large necrotic areas. Sometimes the productive changes are subjected to fibrosis.

Binford (8), while conducting a particular histopathological

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Amphotericin B was used, with great success, in combination
with sulfadiazine in treatment of experimentally infected hamsters.
According to Emmm and collaborators(5), substantially smaller quantities
of lesions in the lungs were observed in the course of such therapy as
well as the prolongation of the survival of experimental animals.
A similar report was given by Louria and coll. (58), applying amphotericin
B to infected white mice.

The first tests of amphotericin B application in human histo-
plasmosis indicate that this antibiotic will produce good results.
Lehan and coll. (53), applying amphotericin B orally in doses of
2-5 grams daily during 2 - 3 months, observed substantial alleviation
of clinical symptoms, and in some patients, remarkable improvement.
Results of the therapy were much better in cases of fresh infections
than in old infections in which substantial organic lesions had
developed.

Good results were likewise obtained by Ellis and coll. (20) and
Zimmerman (87), applying ethyl ester of vanillic acid in skin histo-
plasmosis and in mucous membranes of the larynx, as well as Christie
(11) in case of disseminated histoplasmosis.

In addition to the etiopathogenetic therapy which to date in
many cases does not give the desired effects, a symptomatic therapy
is applied as are surgical operations of resection of the transformed
parts of pulmonary tissue.(47, 59, 71).

Diagnostics of Histoplasmosis

Because of the lack of characteristic clinical symptoms in the
course of histoplasmosis, the entire weight of proper diagnosis is
based on mycological, serological and allergic examinations. Familiarity
with the epidemiological state of the matter as well as the epidemic in-
vestigation in a given case under observation is essential.

In a schematic approach, the course of diagnostic procedure is
the following: first, a skin test with histoplasmin is taken. It permits
to confirm or reject the infection which has already passed or is still
taking place, with the exception of cases which will be discussed later.
Secondly, the isolation of the parasite from the suspected material
is aimed at simultaneously. The following specimens are most frequently
taken: sputum, blood, bone-marrow, pus, exudates, parts of tissues
obtained through biopsy and autopsy, as well as samples of soil, dust
and specimen from animals which were in the environment of the examined
case. From some of the above mentioned specimens, i.e., blood, bone-
marrow, tissue sections, preparations are made immediately, which are
dyed by the Wright or Giemza method and characteristic forms of the
parasite within cells are sought through microscopic examination.

The culture of Histoplasma Capsulatum from infected material
is developed in many media and under various conditions. The lack of
standardization for the methods of fungus isolation in this case
reflects well the difficulties which are faced here. Various centers
and the laboratory give their own substantially modified media and
conditions under which isolation is to be conducted. Therefore, in
order to exploit to the maximum the chance of feeding the parasite,
the cultures are conducted in two temperatures: in 37° and in room
temperature as well as in Sabouraud media and other media containing
extracts from liver, spleen, brain, with the addition of human blood,
horse blood or from other animals. Undoubtedly the best medium con-
stitute those containing extracts from internal animal organs or
blood ingredients. For example, Howell (40), examining the productivity
of isolation from experimentally infected guinea pigs, confirmed
positive cultures of 76% on media containing extracts from brain and
heart, and 10% defibrinised horse blood through incubation in room
temperature. Using another medium and developing cultures in two
temperatures (37° and room temperature), he was able to obtain 100%
positive inoculation.

The growth of the micro-organism, especially in the mycotic
phase, is very slow. It is, therefore, necessary to prevent the
media from drying out and from being overgrown by other, fast-
developing micro-organisms. This difficulty can be eliminated to
a certain degree through the addition of penicillin and streptomycin
in the proportion of 20 to 40 units to 1 ml of medium. The culture is
considered negative only after three or four weeks of observation.
On media containing large quantities of albuminous and sugary components,
the growth takes place generally in the form of mycelium of small
quantity or in complete lack of chlamydospores, indispensable to the
identification of the fungus. It is necessary in these conditions to
transpose the culture on media containing potato flour, agar and
glucose, in order to confirm the presence of spores and especially
chlamydospores.

In spite of the improvements in fungus isolation methods,
confirmation of histoplasmic infection in man through positive culture
is very difficult. According to Curry and Wier (17), for example,
in 65 cases of undoubtful histoplasmosis of the lungs confirmed by
finding of the fungus in resected tissues, Histoplasma Capsulatum was
isolated in only one case. Isolations are more difficult when the
disease has been prolonged. When examinations are made in the early
stages of infection - two, three weeks from the moment of infection
and when the disease has a more acute course, in the disseminated form,
the frequency of positive isolations increases substantially.

A great advancement in the diagnostics of histoplasmosis was
the introduction by Moffit and coll. (61) of the infecting of

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experimental animals with the examined matter. White mice proved to
be especially useful in this case. By peritoneal introduction of
adequately prepared, examined specimens, together with antibiotics,
the frequency of parasite isolation was substantially increased.
The mouse, very sensitive to infection by Histoplasma Capsulatum, in
this case fulfills the role of a filter which eliminates the impurities
of the examined matter with other saprophytic fungi. The infection
of the mouse is a sensitive method, it allows to detect minimal
quantities of fungi; its essential defect, however, is the prolon-
gation, almost for a month, of the time of isolation of Histoplasma
Capsulatum.

Serological reactions, especially the fixation reaction with
various antigens obtained from the yeasty phase and mycotic Histoplasma
Capsulatum, have also a great value for the diagnosis.

Immunological Phenomena in Histoplasmosis

As a result of histoplasmic infection, there appear in the
attacked organism processes aiming at elimination of the invasion of
the pathogenetic factor, conditioned by phenomena of resistivity
and growing immunity. The essence of resistivity in the course of
histoplasmosis is, except for very general and not much examined facts,
little known. According to Beamer (7), it is associated with the
following mechanisms:
1. The natural tissue barrier which prevents the penetration
of micro-organism.
2. Production of substances which block the development
or kill the parasite before its entrance into tissues.
3. Cell protection of the RES system.
4. The increased flow of lymph and blood through the
areas changed by inflammation.
5. Humoral protection (fibrin, probably natural antibodies).
6. A series of environmental conditions, which may not be
favorable to the development of micro-organism; for example: temperature,
pH, content of oxygen, surplus or lack of metabolites, etc.

In immunological phenomena in the course of histoplasmosis
the moment of appearance of allergy on the matter produced from the
cell disintegration of the mycotic phase of Histoplasma Capsulatum,
i.e., on histoplasmin, is essential. Histoplasminic allergy develops
in the second or third week of the disease (54) and lasts through many
years, probably throughout the entire life. Only in cases of sub-
stantial anergy of the organism in the form of disseminated histo-
plasmosis, causing quick death, and in final stages of protracted
mycosis with visible organic changes, is the lack of oversensitiveness
observed. Allergy to histoplasmin like allergy to tuberculine in
tuberculosis, is accepted as proof of the decrease of sensitivity to

recurrent histoplasmic infection. Wilson (85) explains the increase in number of patients suffering from histoplasmosis among the old people by the extinction of the allergic reaction.

The histoplasmic test depends on intradermal introduction of 0.1 ml - adequately diluted, mostly 1/1000 or 1/100, histoplasmin. Results can be studied after 24 and 48 hours. Infiltration in diameter equal to or greater than 5 mm is considered as a positive result.

Histoplasmin is not characterized by great specificity. In other mycoses, coccidioidomycoses and blastomycoses, histoplasmin yields equally positive results. This fact depends on the presence of common allergenic fractions in histoplasmin, coccidioidin and blastomycin.

Interpretational difficulties, in results obtained from dermal reactions are being solved by simultaneous testing with changed mycotic allergenes. This reaction, which forms under the influence of the most diluted allergen, is accepted as specific. There is suspicion that in some areas of U.S.A. other, unknown mycoses appear which can yield positive results with histoplasmin, coccidioidin and blastomin. For example Palmer and coll. (68) indicate that within some states (of U.S.) frequently doubtful results with histoplasmin and coccidioidin are confirmed; this can raise the suspicion that the infection is caused by a different kind of fungi.

Many scientists, using the method of gradual purification of preparations, attempted to eliminate the phenomenon of histoplasmin reaction, not only in histoplasmosis but also in other mycoses. Until now however, a substance characterized by complete specificity and adequate sensitiveness has not yet been obtained from histoplasmin. Dyson and Evans (25), basing their examination on the fact that Histoplasma Capsulatum appears in infected organism exclusively in the yeasty phase, aimed at obtaining from that particular phase adequate allergic preparations. Somatic antigens, extracted from the tissue phase of Histoplasma Capsulatum, were, however, unsuitable for allergic tests. It was possible, however, to obtain allergic substances of great specificity and sensitiveness (greater than those peculiar to histoplasmin) through deduction of precipitates from the filtrates of fluid culture of the yeasty phase with alcohol. Dyson and Evans (25), testing their new substance on pigs experimentally infected with Histoplasma Capsulatum, Coccidioides immitis, Cryptococcus neoformans, Blastomyces dermatitidis, Candida albicans and Sporotrichum schenckii, confirmed the presence of sacral reactions only in animals infected with American drozdzea (trans. note - drozdze - yeast).

In the beginning, skin tests were conducted with various preparations of histoplasmin, which was responsible for many interpre-

tational difficulties and did not allow for comparison of results
obtained during investigations. Thanks to the studies of Shaw and
coll. (81), it became possible to avoid these divergencies; namely,
in 1950 the standard preparation of histoplasmin H-15 was introduced
to massive application. This histoplasmin, up to the present, has not
lost any of its value and it serves as an example for other histo-
plasmin preparations. Histoplasmin is a filtrate of several months
culture duration on a synthetic medium of the mycotic phase of
Histoplasma Capsulatum. It is composed of polysaccharide and
albuminous fractions. The elimination of the albuminous part does not
deprive histoplasmin of the ability to react in skin reaction (16).
The full histoplasmin serves as an antigen in the fixation reaction,
precipitation and hemagglutination. However, in serological reactions,
histoplasmin shows a great degree of non-specificity of reaction.
Therefore, in diagnostic examinations, in the search for antibodies
in examined serums, except histoplasmin, other antigens from the yeasty
and mycotic phases of Histoplasma Capsulatum are also applied.

The fixation reaction has a great significance for diagnostics
as well as for prognostics of histoplasmosis.

The antibodies taking part in this reaction, appear in the
third or fourth week of the disease and remain in serum from several
months to several years. A characteristic feature is the high titer
of antibodies in the disseminated forms of histoplasmosis. In the
mild form of the disease, antibodies are not found, or they appear in
small quantity. The titers of antibodies reach their maximum in the
fifth - sixth week of the disease. Sometimes, they can be found in
the dilution of serum 1 to several or even several thousands (46,80).
There is a great probability, based on examinations in the course of
coccidicidomycosis as well as on few observations in histo-
plasmosis, that the quantity of antibodies present in serum, taking
part in the fixation reaction, is directly proportional to the
quantity of active cells of the parasite in the organism.

Quite an essential problem in the discovery of drucnyunikow
(trans. note - histoplasmosis ?, antibodies, active cells?), is the
matter of using the proper antigens. Experiments with a series of
nuclei in the fixation reaction indicate that it is erroneous to apply
only one antigen to discover antihistoplasmic antibodies (46). It is
recommended to use antigens obtained from cells of the yeasty phase
and from culture filtrates of both phases. This can be deduced from
the fact that in the course of various periods of the disease and in
various cases of histoplasmosis, the presence of antibodies for only
one or a second antigen is sometimes confirmed.

The problem of why and when certain antibodies appear, has
not yet been solved. Labofficky and coll. (50) while concerning

themselves with these problems showed, that for eight antigen fractions
obtained by them from Histoplasma Capsulatum, the antibodies in sera
of immunized rabbits appear in various periods of time. The anti-
genic fractions IV, VI and IX distinguished themselves by a clear
specificity, the rest reacted with homological sera as well as with
sera of rabbits infected with other diphased fungi. Similar examinations
made with human sera give an opportunity to introduce specific antigen
for discovery of antihistoplasmic antibodies. Such antigens would be
characterized not only by great specificity, but would allow to determine
the time of infection, the course of the disease and the prognosis. (50).

The precipitatory reactions have a great significance for the
identification of histoplasmosis, especially in the early stages. Such
a reaction was applied for the first time, by Van Pernis and coll.(84),
and later by Schaff (79), Pates. (69), as well as by Salvin and Hottle
(75). The latter confirmed that precipitines, in the course of
experimental histoplasmosis, show up shortly after the appear.ance
of allergic reaction and maintain themselves in short-lasting sera.
Observations made by Salvin and Hottle (75) were confirmed several
years later on humans. (77). Precipitative reactions with histo-
plasmin come out positively in the first or second week of the
disease and remain in serum from 3 weeks to 10 months.

By comparing the results of the fixation reaction and pre-
cipitation, no correlation between them was confirmed. The precipitation
reaction is especially helpful for identification of acute cases of
histoplasmosis, and those cases in which the fixation reaction was
negative. The precipitative tests with histoplasmin come out positively
not only with sera of histoplasmosis cases, but also in other mycoses.
Titers, however, in specific reactions are substantially higher and
they make possible the actual identification of the pathologic unit.

Recently, the precipitation reaction in agar gel according to
Ouchterlon was introduced to the diagnosis of histoplasmosis. Thanks
to this technique, Heiner (45) gained with positive human sera and
histoplasmin two precipitative lines of which one is characteristic
to the pathologic process, and the other only proves the existence
of the state of supersensitiveness to histoplasmin.

An attempt was also made to introduce the reaction of passive
agglutination to the discovery of antihistoplasmic antibodies. Colloid
particles (78) and red blood corpuscles (63) sensitized with histo-
plasmin were applied to the reaction. This method, however, was not
commonly accepted.

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